Remarks and Arguments

Claims 62-63, 65-66, 70-73, 75-79, 82, 87-90, 99-100, 102-110, 113, 118-125, 131 and 133 are pending. Claims 62, 65, 66, 82, 99, 113, 121, 122, 131 and 133 are amended in response to the Examiner's comments. Applicants reserve the right to present additional subject matter in one or more continuation or divisional applications.

Double Patenting

Applicants note that the Examiner has provisionally rejected claims 62-63, 65-66, 70-73, 75-79, 82, 87-90, 99-100, 102-110, 113, 118-125, 131 and 133 on the grounds of nonstatutory obviousness-type double patenting over claims 62-69, 71-86 and 88-97 of copending Application No. 11/641,644. Applicants note that the 11/641,644 application is later filed than the present case and request that the issue be addressed in the later filed case.

New Matter

Applicants note that the Examiner has rejected claims 62-63, 65-66, 70-73, 75-79, 82, 87-90, 99-100, 102-110, 113, 118-125, 131 and 133 under 35 U.S.C. §112, first paragraph as including new matter for recitation of the term "fibroblast-like."[1] To facilitate prosecution, Applicants have removed the term "fibroblast-like" from the claims. However, Applicants again note, as apparently accepted by the Examiner, that the term "fibroblast" is an art recognized term and that the art was concerned only with the morphological characteristics of the cells used in nuclear transfer rather than to a characterized cell lineage. Specifically, that is how the term "fibroblast" is used in the present specification, i.e., to describe cells that are *morphologically* fibroblast-like.[2] The present claims to identify "fibroblasts" as the cell type being modified in step (a) consistent with that art-recognized view of the term.[3]

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[1] Applicants had understood this term to be the Examiner's suggestion, as, at page 4 of the previous Action, the Examiner pointed out that "[t]he art recites using fibroblast, or fibroblast-like cells."

[2] Applicants do not dispute that true fibroblasts have certain genetically determined characteristics, only that these are not confirmed in cells used for nuclear transfer. As noted in the previous responses, methods of preparing cells for nuclear transfer involve taking a tissue, dispersing the cells and growing these *in vitro.* Applicants are aware of only one study in this field in which *any* further investigation as to the cellular identity of the cells was conducted (Onishi, et al. (2000) *Science* 289:1188-1190)). Remaining studies describe the culturing of cells without analysis or identification by anything other than morphological characteristics (see e.g. Lai, et al. (2002) *Science* 295:1089-1092 and Dai, et al. (2002) *Nature Biotech.* 20:251-255). Furthermore, as the Examiner is aware, the culture conditions in which the cells are grown can induce a fibroblast-like morphology (Kalluri and Neilson (2003) *J. Clin.*

Rejection under 35 U.S.C. §112, first paragraph, enablement

The Examiner had previously acknowledged that the art recites using fibroblast or fibroblast-like cells (see Office Action of September 18, 2008, page 4), in response to which the Applicants amended the claims to recite "fibroblast-like." The Examiner has now rejected the claims as lacking enablement under 35 U.S.C. §112, first paragraph for the recitation of fibroblast-like. The Examiner has noted that the Applicants "have not provided any guidance as to which qualities/characteristics of fibroblasts a cell must have" in order to be fibroblast-like. As noted above, the claims have been amended to remove this term. Applicants believe this amendment addresses the Examiner's rejection and ask for its withdrawal.

Rejection under 35 U.S.C. §112, second paragraph

The Examiner has rejected the pending claims under 35 U.S.C. §112, second paragraph as indefinite for recitation of the term "fibroblast-like." As noted above, the claims have been amended to remove this term. Applicants believe this amendment addresses the Examiner's rejection and ask for its withdrawal.

Rejections under 35 U.S.C. §102(b)

The Examiner has again rejected claims 62-63, 65-66, 75-76, 82, 87-90, 99-100, 106, 113, 118-122, 131 and 133 under 35 U.S.C §102(b) as anticipated by Campbell, et al. (WO 97/07669) or under 102(e) as anticipated by U.S. Patent No. 6,147,276 (also Campbell).

The Examiner has asserted that Campbell "provides each and every step required by the claim" and thus anticipates the invention. Specifically, the Examiner has stated that "Campbell teach that transgenic animals can be made, by using a cell such as a fibroblast. This is exactly

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Invest. 112:1776-1784). Therefore, it is likely that many of the cells that resemble fibroblasts in culture did not originate as such and instead carry markers for their cell type of origin. The art recognizes this complexity, as supported by Chang et al. who note that "[i]t is now clear that the cells we traditionally call fibroblasts comprise a diverse class of distinct differentiated cell types…" (see Chang, et al. (2002) *PNAS* 99:12877-12882, page 12882, emphasis added).

[3] The Applicants use of the term "fibroblast" is consistent with the use of the term in the art. Denning et al. *specifically* note that the "fibroblast cells" discussed throughout the paper are identified as such "[b]ased on morphological appearance…" (Denning, et al. (2001) *Cloning Stem Cells* 3:221-231) and Wilmut et al. describe the cloning of sheep using what is termed in Figure 1 a population of "fetal fibroblasts (BLWF1)" even though, in the description of the methods of isolation of the cells, the authors described these same cells merely as "fibroblast-like." (see Wilmut et al. (1997) *Nature* 385:810-813 (compare Figure 1b to page 813, top left))

what is claimed." Applicants note that this not an accurate statement. In particular, Campbell *does not* provide the steps recited in the amended claims including: a) that any genome be targeted by homologous recombination; b) that homologous recombination occur at an endogenous locus; or c) that the genome have a normal karyotype.

The steps that are lacking in Campbell are not merely "inherent" in any recitation of genetic modification. Indeed, it was well recognized in the art that a number of methods could be used for genetic modification. Applicants attach hereto, as an example, Kucherlapati (1986) "Gene Transfer" *Plenum Press*, the table of contents of which provides an overview of the many different types of techniques that were used to provide genetic modification in cell lines prior to the present invention. The present Applicants were the first to show that the specifically recited technique that includes homologous recombination at an endogenous locus in an animal with a normal karyotype would produce a viable, genetically targeted animal. This is neither discussed nor exemplified in Campbell. Because the claims differ from what was disclosed in Campbell, Applicants respectfully request withdrawal of the rejection under 35 U.S.C. §102(b) and 102(e).

Although the Examiner has not specifically renewed the prior rejection under 35 U.S.C. §103 over Campbell, the Examiner did respond to the arguments presented in the prior response against the rejection under 35 U.S.C 102/103, but apparently misunderstood these arguments. Applicants previously argued that Campbell did not teach the presently claimed methods, and that, to the extent that the Examiner relies on the "ordinarily skilled artisan," such an artisan would have had no expectation of success in <u>combining</u> the nuclear transfer techniques described in Campbell with genetic targeting of somatic cells otherwise known in the art. The Examiner has responded to these arguments by asserting that each of the references cited by the Applicants individually "does not show that somatic cells <u>cannot be targeted</u>" and that "the art, in no way, teaches away from producing a somatic cell with a specific genetic targeting event." (see Office Action pages 10-12).

Applicants respectfully point out that <u>somatic cell targeting is not the invention.</u> Rather, the invention involves the combination of somatic cell targeting with nuclear transfer to produce viable animals. Applicants remind the Examiner that the invention cannot be parsed into its part. The invention <u>as a whole</u> must be addressed in any rejection. The Examiner's assumption that the successful use of homologous recombination in somatic cells *in vitro* somehow

correlates with its use in producing viable animals is inconsistent with the evidence provided by the Applicants. Specifically, the Applicants have provided:

a) evidence that supports that homologous recombination in somatic cells occurs at much lower frequency in somatic cells than in embryonic stem cells (see see para 0117, also Arbones, et al. (1994) *Nat. Genet.* 6:90-97) and that primary somatic cells have a lower frequency of homologous recombination than immortalized cells (see para 0118, also Finn, et al. (1989) *Mol. Cell. Biol.* 9:4009-4017 and Thyagarajan et al. (1996) *Nuc. Acids Rsch.* 24:4084-4091)

b) evidence that fibroblasts undergo "crises" of senescence when cultured for extended periods (see Denning, et al. (2001) *Cloning and Stem Cells* 3:221-231and Thomson, et al. (2003) *Reprod. Supp.* 61:495-5008,[4] for discussion)

c) evidence that somatic cells that have been cultured for extended periods were believed to be too compromised to produce viable animals (see e.g. Porter, et al. (1997) *Transplant.* 64:1227-1235, Piedrahita (2000) *Transgenic Research* 9:261–262, Suraokar and Bradley (2000) *Nature* 405:1004-1005, and the declaration of Dr. Azim Surani).

The Applicants have never argued that somatic cell targeting could not be achieved. What Applicants have argued, repeatedly, is that somatic cell targeting, as it was known in the prior art, was recognized as an inefficient process that could not be combined with nuclear transfer to produce viable animals with any expectation of success. The Applicants have recited a specific method of modification, to a specified type of genome, none of which is provided in Campbell.

The Examiner has failed to provide any support as to why a person of ordinary skill in the art, prior to the present invention, would have had any "reasonable expectation" that a viable animal with a targeted genetic modification could be successfully produced via homologous recombination of fibroblast cells and subsequent nuclear transfer cloning, as presently claimed. Indeed, even the art cited by the Examiner against the enablement of the present claims does not support this statement.

[4] cited by Examiner in related Application No. 11/641,644

It was accepted in the art, both before and after Campbell, that there would be extreme difficulties in providing somatic cells with targeted genetic modifications *that were still suitable for nuclear transfer*. Although it was known that somatic cells could be targeted *in vitro*, because of the low efficiencies of this process it was expected that any somatic cell would have to be cultured for extended periods to identify a targeted event, particularly when targeted by homologous recombination.[5] The specification *and* the art support that it was a well established belief that viable animals with targeted genetic modifications *could not be produced* using nuclear transfer because the somatic cells would be too compromised by this extended culturing.

Even shortly after the filing of the present case, authors such as Piedrahita note that available techniques were not likely to lead to viable animals with targeted genetic modifications, and that "in the future" techniques may be found for producing such targeted animals.[6] This is also supported in Porter et al. who noted that "[t]he development of pigs carrying targeted mutations in genes has not been explored because, as yet, the technology to make such animals does not exist."[7] Until the date of the present invention therefore, skilled artisans were convinced that making viable, genetically targeted animals using nuclear transfer was not within their skill.

Nothing in Campbell provides any evidence that would overcome this conviction. Indeed, Campbell even references the failure of using donor cells that had been in "prolonged culture" in nuclear transfer techniques.[8] Campbell's discussion of gene targeting is merely a broad note that techniques "may" be utilized with no direction to what specific techniques, on what types of cells, would provide the hoped for result, offering nothing further to overcome the art recognized problems. Furthermore, one of ordinary skill in the art reading Campbell would have recognized that the cells specifically described *could not* have been used in the presently claimed methods that require homologous recombination and subsequent nuclear transfer

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[5] Applicants note that the mere ability to grow a cell in culture, or even to use such a cell in gene therapy, does *not* support that such a cell is suitable for use as a nuclear donor to provide a viable animal. The problems expected to occur in somatic cells during extended culturing, prior to the present invention, are made clear in the Surani declaration.

[6] see Piedrahita (2000) *Transgenic Research* 9:261–262

[7] see, e.g. Porter, et al. (1997) *Transplant.* 64:1227-1235, see page 1233, left column. This perceived difficulty is highlighted in Suraokar and Bradley (2000) *Nature* 405:1004-1005who note that it was recognized that "[f]ibroblasts cannot proliferate for long *in vitro*, and acquire chromosomal changes quite quickly" and note that "[u]ntil [the present invention], no one had shown that it would be possible to specifically modify endogenous genes" and produce genetically targeted livestock by nuclear transfer.

[8] see pages 2 and 3 of Campbell

cloning, further supporting that one of ordinary skill would not have read Campbell as inherently describing the presently claimed methods. Specifically, Campbell points to Robertson, E.J. for isolation of primary fetal fibroblasts.[9] Applicants attach a copy of the relevant pages of this reference for the Examiner and point specifically to page 77, Section 2.4.2 entitled "Preparation Of Feeder Layers From Primary Embryonic Fibroblasts" where it is noted that "a major disadvantage [of these cells] is the limited culture lifespan of primary cells; they become senescent after about 15-20 cell divisions" (emphasis added).[10] As noted in Clark, et al., it was understood that primary cultures had to be expanded for at least 15 population doublings to even allow for transfection, let alone confirmation of a targeted integration. Indeed, obtaining targeted cells required more than two weeks of in vitro culture, and, according to Clark's estimate, at least *45* population doublings to generate targeted cells for nuclear transfer.[11]

Given these facts, anyone of ordinary skill would have expected that any somatic cells described in Campbell would already be in the process of senescence by the time of transfection. Because genetic modification by homologous recombination is a highly inefficient process (as discussed above), anyone of skill in the art would have expected that the cells would have to be further selected after transfection to obtain a targeted cell. Given the understanding of somatic cell senescence as discussed in Robertson, on of ordinary skill prior to the present invention would have expected the possibility of finding a targeted cell without further selection would be so remote as to be negligible. Given that a person of ordinary skill in the art had no expectation of finding a cell with a targeted genetic modification that was still viable and had a normal karyotype suitable for successful nuclear transfer, no one of ordinary skill would have undertaken such a project.

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[9] Robertson, E.J. in Teratocarcinomas and Emrbyonic Stem Cells: A Practical Approach, IRL Press Oxford (1987) p 71-112 (see page 32, lines 13-17 of Campbell)

[10] Applicants note that the "Hayflick" effect, which describes the limited lifespan of cells in culture, was a well recognized phenomenon many years prior to the present invention. The Hayflick effect is attributed to telomere attrition during each round of replication, which effectively "ages" the cells, causing them to senesce over a period of time in culture. A person of ordinary skill would not have expected somatic cells in extended culture to be capable of creating a viable animal because they would have been subject to this effect.

[11] Clark, et al. (2000) Transg. Rsch. 9:263-275, see page 268. This is further supported by the work done on embryonic stem cells, in which primary cultures are grown for between 12 and 18 population doublings before being genetically manipulated, let alone before screening could be carried out (see Joyner, ed. (1995) "Gene Targeting: A Practical Approach" Oxford U. Press, pages 48-49 and Robertson, p. 102 (for doubling time)). As noted, embryonic stem cells have higher transformation efficiencies than somatic cells, therefore targeted embryonic stem cells would require fewer passages as compared to even the best somatic cells.

The Applicants have thus provided ample evidence supporting the *lack* of expectation of success by anyone of ordinary skill in the art that would cause such a person to avoid combining the known technique of somatic cell targeting via homologous recombination with Campbell's description of somatic cell nuclear transfer. Indeed, the Examiner has provided no evidence that there was any expectation that existing techniques could be used to make such animals, given the failure of extended somatic cell culturing highlighted by authors such as Denning. Given that Campbell neither discloses nor suggests the claimed methods, and that one of ordinary skill would not consider Campbell to have 'inherently' or 'necessarily' described these methods, Applicants respectfully request withdrawal of the rejections under 35 U.S.C. §102(b) and 102(e).

Rejections under 35 U.S.C. §103

The Examiner has rejected claims 62, 63, 65, 66, 75-79, 82, 87-90, 99-100, 106-110, 113, 118-124, 131 and 133 under 35 U.S.C. §103(a) as obvious over Campbell in view of d'Apice, et al. (U.S. Patent No. 5,849,991). The Examiner asserts that the claims "recite modifying an endogenous locus by a genetic targeting event" and that this could mean either random or non-random integration. Although Applicants are puzzled as to this interpretation of the claims, given that the term "targeting" is fairly straightforward, the amended claims recite homologous recombination and therefore overcome this interpretation.

The Examiner has asserted that d'Apice is not relied on for nuclear transfer but for producing animals, including but not only, mice, lacking α(1,3)galactosyltransferase. As noted above, Campbell did not disclose or suggest the presently claimed methods. Although it was known in the art that somatic cells, and particularly fibroblast cells, could be genetically modified using homologous recombination, it was also well known that these cells were subject to senescence and related changes that made it unfeasible to combine nuclear transfer as taught by Campbell with somatic cell homologous recombination. D'Apice provides no additional support to overcome this deficiency. As previously discussed, d'Apice relates in its entirety to embryonic stem (ES) cells. Somatic cells and embryonic stem cells are not the same (as discussed in the present specification). The techniques described in d'Apice, as well as any other reference dealing with production of chimeric animals using ES cells, differ dramatically from somatic cell nuclear transfer. Although transgenic animals had been successfully produced using ES cells prior to the present invention, ES cells do not exhibit the low frequency of

integration, senescence and other cell culture related changes that are exhibited by somatic cells (see Suda, et al. (1987) *J. Cell. Physiol.* 133:197-20, abst.). Furthermore, the techniques used by d'Apice and others using ES cells do not involve nuclear transfer and provide no additional support for the presently claimed methods. Applicants respectfully request withdrawal of this rejection.

The Examiner has similarly rejected claims 62, 63, 65, 66, 70, 73, 75-77, 82, 87-90, 99-100, 102, 105-108, 113, 118-122, 125, 131 and 133 under 35 U.S.C. §103(a) as obvious Campbell in view of Kucherlapati et al. (WO 94/02602). The Examiner asserts that Campbell is used to provide guidance for producing cloned, transgenic animals and apparently asserts Kucherlapati as providing motivation to knock out certain genes. As noted above, the mere motivation to provide transgenic animals is insufficient to remedy the defects of Campbell. Kucherlapati, like d'Apice, relates to embryonic stem cells that are not used in somatic cell nuclear transfer. The same problems that are associated with genetic modification of somatic cells *do not exist* in embryonic stem cell technologies.

The Examiner asserts that this argument fails because the claims are not limited to large animals. Applicants respectfully point out that the argument is as to the question of whether d'Apice or Kucherlapati provides any guidance in providing a targeted genetic modification of somatic cells <u>in combination with nuclear transfer</u>. Since the techniques of d'Apice and Kucherlapati are related to ES technology and *not* to somatic cell nuclear transfer, they do not. Applicants respectfully request withdrawal of this rejection.

The Examiner has rejected claims 62, 63, 65, 66, 70, 72, 75, 76, 82, 87-90, 99-100, 102, 104, 106, 113, 118-122, 131 and 133 under 35 U.S.C. §103(a) as obvious in view of Campbell and U.S. Patent No. 6,013,857 ("the '857 patent"). The Examiner asserts that it would have been obvious to modify the methods taught in Campbell to place a transgene of interest adjacent to an endogenous promoter, such as a milk promoter, with a reasonable expectation of success. Applicants note that the '857 patent provides no more direction to making a targeted genetic modification in fibroblast cells by homologous recombination and combine this with somatic cell nuclear transfer than any other reference cited. The entire patent is directed at using ES techniques or oocyte injection in cows and there is no exemplification of any other technique

useful to achieve the claimed method. This is apparent in the abstract which cites that "[t]he method includes introducing the above transgene into an embryonal target cell of a bovine species..." Much as with the other cited references, the '857 patent provides no additional guidance to supplement Campbell to reach the methods recited in the amended claims.

The Examiner has also rejected claims 62, 63, 65, 66, 70, 71, 75, 76, 82, 87-90, 100, 102, 103, 106, 113, 118-122, 131 and 133 under 35 U.S.C. §103(a) over Campbell in view of Bedalov (1994) *J. Biol. Chem.* 269:4903-4909 taken together with Rossert (1995) *J. Cell Biol.* 129:1421-1432. The Examiner asserts that Bedalov discusses the COL1A1 promoter fused to a reporter gene in a variety of mesenchymal cell types and that Rossert teaches that the precise sequences responsible for lineage specific expression had not been defined. Again, none of the additional references provide any teaching to overcome the deficiencies of Campbell.

None of the cited references, alone or in combination overcome the lack of expectation of success for combining specific known techniques to produce viable, genetically modified animals. The present invention was the first in the world to produce such animals, which had long been desired but never made. The Applicants found that a method of modification of certain somatic cells in which the genome has a normal karyotype using homologous recombination and subsequent use of these cells in a specific nuclear transfer method could be used to successfully produce a viable animal. No one of ordinary skill in the art would have combined the techniques of homologous recombination and somatic cell nuclear transfer, as recited in the claims, because of the well-recognized expectation that somatic cells would senesce and be unusable for creation of a viable animal after being subject to homologous recombination. Applicants respectfully request withdrawal of the rejections under 35 U.S.C. §103(a).

The Commissioner is hereby authorized to charge any underpayment or credit any overpayment of fees to Deposit Account No. 11-0980.

Respectfully submitted,

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